colony forming units ("CFU") per milliliter ("ml.") of water and comprises the following steps:

(a) culturing an identified *Legionella* bacteria species, or serogroup of a species, to a desired size and harvesting therefrom cells of that species, or serogroup of a species, as a wet cell pellet;

(b) obtaining from the wet cell pellet an essentially protein-free carbohydrate antigen by a series of steps which comprises

(i). suspending the wet cell pellet in an alkaline solution and mixing;

(ii). adjusting the pH to an acid pH with a strong acid;

(iii). separating the mixture from step (ii) into two layers;

(iv). removing the upper layer and adjusting its pH to approximate neutrality;

(v). adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi). adjusting the pH of the product from step (v) to the alkaline side with a weakly alkaline aqueous solution and;

(vii). separating out an essentially protein free carbohydrate antigen;

(c) coupling to a chromatographic affinity column through a spacer molecule the essentially protein-free carbohydrate antigen obtained in step (b);

(d) passing antibodies to the *same Legionella* species, or serogroup of a species, as that cultured in step (a) over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

(e) performing an enzyme immunoassay upon a water sample suspected of being infected with *Legionella* bacteria of the same species, or serogroup of a species as that cultured in step (a), which assay comprises the following steps:

(i) coating a solid substrate with antigen-specific antibodies from step (d) hereof in an amount sufficient to provide a coating containing at least 0.05 μg per test of said antibodies and allowing the coated substrate to dry;

(ii) preparing a conjugate of an enzyme and antigen-specific antibodies from step (d) hereof;

(iii) bringing the sample to be tested and from 0.2 to 2.0 μg conjugate per test into contact with buffer solution and the coated solid substrate of step (i) in a suitable reaction vessel and incubating for a period of at least 20 minutes; and

(iv) decanting liquid from said vessel, washing, adding a colorimetric, chemiluminescent or bioluminescent material thereto, allowing color, chemiluminescence or bioluminescence to develop for up to about 5 minutes, measuring its intensity in any known manner and determining therefrom the concentration of the suspected *Legionella* bacteria species or serogroup of a species in the original water sample according to predetermined intensity/concentration standards which first correlate the intensity to the amount of O-carbohydrate antigen detected in the

sample and correlate that in turn to the concentration of bacteria in CFU/ml in the water being tested.

11 The method of claim 10 in which the *Legionella* bacteria species, or serogroup of a species, in step (a) is a serogroup of *Legionella pneumophila* bacteria and the raw antibodies of step (d) are raw antibodies to bacteria of the same serogroup of *Legionella pneumophila*

12 The method of claim 11 in which the bacteria of a serogroup of *Legionella pneumophila* are bacteria from serogroup 1.

13 The method of claim 11 in the which bacteria of a serogroup of *Legionella pneumophila* are bacteria from serogroup 5.

14 The method of claim 10 in which the spacer molecule of step (c) is a protein molecule.

15 A method according to claim 12 wherein the enzyme immunoassay is run as a sandwich assay.

16 A method according to claim 12 wherein the enzyme immunoassay is run as a competitive assay.

17. A method according to claim 10 wherein the coated solid substrate in step (e) (i) is the inner wall of a test tube, which test tube also serves as the test reaction vessel in step (e) (iii)

18 A method according to claim 10 wherein the coated solid substrate of step (e) is selected from among coated solid inserts and coated beads.

19 A method according to claim 10 wherein the enzyme reacted in step (e) (ii) is horseradish peroxidase and a colorimetric agent, tetramethylbenzidine, is added in step (e) (iv).

20 A method according to claim 10 wherein the water to be tested is water obtained from the heating and/or cooling system of a building, or water obtained from a building sanitation or drinking water supply, individual water samples are obtained in quantities of from 100 to 1000 ml. per test and each individual water sample is subjected to a pre-assay concentration step.

21 A method according to claim 20 wherein the concentration step comprises filtering each individual water sample through a filter having a pore size not greater than 0.45μm, and it is followed by collecting filter residue by thoroughly stroking the filter with the swab pad of a swab comprising a handle and an affixed pad of fibrous material or foamed open pore material , and delivering the sample on said swab pad to the test receptacle of step (e) (iii) by immersing the swab pad in the buffer solution contained therein, twirling the pad in said solution and leaving the pad immersed therein throughout the period of incubation set forth in step (e) (iii).

22 A method according to Claim 21 wherein the buffer solution is composed of aqueous 0.05 M tris HCl containing 2-5% of a detergent having a pH of 7.0

23 A method according the claim 20 wherein the concentration step comprises subjecting each individual water sample to high speed centrifugation followed by settling and removal by decantation or aspiration of supernatant water and it is followed by thoroughly stroking the residual solids with the swab pad of a swab comprising a handle and an affixed pad of fibrous material or foamed open pore material and delivering the sample on said swab

pad to the test receptacle of step (e) (iii) by immersing the swab pad in the buffer solution contained therein throughout the incubation period set forth in step (e) (iii).

24 A method according to claim 23 wherein the buffer solution is composed of aqueous 0.05 M tris H Cl containing 2-5% of a detergent having a pH of about 7.0.

25 A method for determining the concentration of at least one species or serogroup of a species of *Legionella* bacteria in environmental water suspected of being infected therewith, which method comprises the following steps:

(a) culturing an identified *Legionella* bacteria species, or serogroup of a species, to a desired size and harvesting therefrom cells of that species, or serogroup of a species, as a wet cell pellet;

(b) obtaining from the wet cell pellet an essentially protein-free carbohydrate antigen by a series of steps which comprises

(i). suspending the wet cell pellet in an alkaline solution and mixing;

(ii). adjusting the pH to an acid pH with a strong acid;

(iii). separating the mixture from step (ii) into two layers;

(iv). removing the upper layer and adjusting its pH to approximate neutrality;

(v). adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi). adjusting the pH of the product from step (v) to the alkaline side with a weakly alkaline aqueous solution and;

(vii). separating out an essentially protein free carbohydrate antigen;

(c) coupling to a chromatographic affinity column through a spacer molecule the essentially protein-free carbohydrate antigen obtained in step (b);

(d) passing antibodies to the *same Legionella* species, or serogroup of a species, as that cultured in step (a) over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

(e) preconcentrating the sample by (1) adding thereto an aqueous medium containing finely divided magnetizable particles which have been precoated with purified antibodies from step (d) hereof, which antibodies tend to draw to themselves carbohydrate antigens from the sample and to react therewith to form conjugates, (2) subjecting the mixture of sample and magnetizable particles to the action of a local magnetic field, whereby they are caused to form a coherent mass, (3) decanting or aspirating off the water from the coherent mass and (4) subjecting the mass, in a known manner, to demagnetization and then to elution of the antigen-antibody conjugates from the particles; and

(f) performing an enzyme immunoassay upon the resulting eluate according to the following steps:

(i) coating a solid substrate with antigen-specific antibodies from step (d) hereof in an amount sufficient to provide a coating containing at least 0.05 μg per test of said antibodies and allowing the coated substrate to dry;

(ii) preparing a conjugate of an enzyme with the antibody-antigen conjugates in the eluate from step (e);

(iii) bringing enzyme-antibody-antigen conjugate containing from 0.2 to 2.0 μg per test of enzyme antibody content into contact with buffer and the coated solid substrate of step (i) in a suitable reaction vessel and incubating for a period of at least twenty minutes; and

(iv) decanting liquid from said vessel, washing, adding a colorimetric, chemilumescent or bioluminescent material thereto, allowing color, chemiluminescence or bioluminescence to develop for up to about 5 minutes, measuring its intensity in any known manner and determining therefrom the concentration of the suspected *Legionella* species, or serogroup of a species in the original water sample according to predetermined intensity/concentration standards which first correlate the measured intensity to the amount of O-carbohydrate antigen present in the sample and correlate that in turn to the concentration of the *Legionella* bacteria, in CFU/ml, in the water being tested.

26 The method of claim 25 in which the *Legionella* bacteria species, or serogroup of a species, in step (a) is a serogroup of *Legionella pneumophila* bacteria and the raw antibodies of step (d) are raw antibodies to bacteria of the same serogroup of *Legionella pneumophila*

27 The method of claim 25 in which the bacteria of a serogroup of *Legionella pneumophila* are bacteria from serogroup 1.

28 The method of claim 26 in the which bacteria of a serogroup of *Legionella pneumophila* are bacteria from serogroup 5.

29 The method of claim 25 in which the spacer molecule of step (c) is a protein molecule.

30 A method according to claim 27 wherein the enzyme immunoassay is run as a sandwich assay.

31 A method according to claim 28 wherein the enzyme immunoassay is run as a competitive assay.

32 A method according to claim 25 wherein the coated solid substrate in step (f) (i) is the inner wall of a test tube, which test tube also serves as the test reaction vessel in step (e) (iii).

33 A method according to claim 25 wherein the coated solid substrate of step (f) (i) is selected from among coated solid inserts and coated beads.

34 A method according to claim 25 wherein the enzyme reacted in step (f) (ii) is horseradish peroxidase and a colorimetric agent, tetramethylbenzidine, is added in step (f) (iv).

35 A method according to claim 25 in which the buffer solution of step f (iii) is aqueous 0.5 M tris H Cl containing 2-5% of a detergent having a pH of about 7.0.

Inasmuch as the total number of claims herein is now 26, of which two are independent claims and 24 are dependent claims, applicants submit herewith a check for $54.00 to cover small entity fee for each claim in excess of twenty.